

Chromosome Analysis Guidelines Preliminary Report

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PREFACE: *These guidelines have been developed by the Association of Cytogenetic Technologists (ACT) for chromosome analysis. In formulating its recommendations, the task force reviewed guidelines established by several states and regional genetics groups. Draft guidelines prepared by the task force were reviewed by a panel of expert consultants, all of whom are laboratory directors and well known in their respective fields of expertise. The intention of the task force was to reflect procedures that are believed to be generally accepted by cytogenetic laboratories as basic criteria for effective chromosome analysis and that are consistent with existing cytogenetic quality assurance guidelines.*

It is important to stress that the primary purpose of the task force at this time is to establish guidelines for chromosome analysis. While the present guidelines address issues other than chromosome analysis, they do so incidentally and only in general terms. A more comprehensive discussion of other technical aspects of cytogenetics can be found in the forthcoming second edition of the ACT Cytogenetics Laboratory Manual [1].

It is important to note that these guidelines are not intended to prescribe appropriate analyses for all individual circumstances. That determination is appropriately a matter for the judgment of the laboratories concerned. ACT, its members, and the task force that assisted in preparation of these guidelines make no warranty and assume no liability with respect to the information contained herein.

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The following guidelines for chromosome analysis, previously published (Karyogram 15:131-135, 1989) should be of interest to the readers of Cancer Genetics and Cytogenetics, not only because they contain a section on cancer chromosome studies, but also guidelines for a number of other areas of interest to cancer cytogeneticists. Readers are encouraged to send their comments or criticisms to the Chair of the Task Force responsible for putting these guidelines together.

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CHROMOSOME ANALYSIS GUIDELINES

Amniotic Fluid Studies

Flask method.

Count:* 15–20 cells, from a minimum of two independently established cultures.
 Analyze: 4–5 cells.

Karyotype: 2 cells;** in cases with mosaicism, karyotype minimum of 1 cell per cell line.

In Situ Method.

Count:* 15 cells from a minimum of 10–15 colonies, from a minimum of two independently established cultures.

Analyze: 4–5 cells, each from different colonies (preferably from at least two independently established cultures).

Karyotype: 2 cells;** in cases with mosaicism, karyotype minimum of 1 cell per cell line.

Chorionic villus sampling (CVS) studies

The direct method and culture method for CVS both appear to have relatively high reliability, although discrepancies have occurred between CVS results and fetal results with a low frequency (most reported cases concern the direct method). Until further data being collected by the National Institutes of Health collaborative trial are available, we recommend a conservative strategy of analyzing cells from both methods (which assays two different cell types, cytotrophoblasts in the direct, and mesenchymal core in culture) whenever possible.

Direct preparation.

Count:* 15–20 cells, if possible.

Analyze: 4–5 cells, if possible.

Karyotype: 2 cells;** in cases with mosaicism, karyotype minimum of 1 cell per cell line.

Culture.

Count:* 15–20 cells from two independently established cultures.

Analyze: 4–5 cells.

*Noting any numerical/structural aberrations observed.

**These may be 2 of the 4–5 cells analyzed.

Karyotype: 2 cells;** in cases with mosaicism, karyotype minimum of 1 cell per cell line.

Combination of direct and culture preparations.

Count:* a total of 15–20 cells, with at least 10 cells coming from the cultured preparation.

Analyze: 4–5 cells.

Karyotype: 2 cells;** in cases with mosaicism, karyotype minimum of 1 cell per cell line.

Constitutional Studies (Peripheral Blood, Bone Marrow, and Tissue Studies)

Count:* 15–20 cells.

Analyze: 4–5 cells.

Karyotype: 2 cells;** in cases with mosaicism, karyotype minimum of 1 cell per cell line.

Note: It is recommended that bone marrow specimens obtained from newborns, which may be of poor quality, be accompanied by peripheral blood, which can be used to confirm and more accurately define subtle chromosomal abnormalities.

Fragile X Studies

Because fragile X cytogenetic protocols vary considerably, each laboratory offering such analysis should establish written guidelines to include culture methods to be applied, banding methods, cell selection criteria, scoring criteria, and number of cells to be scored.

Recommendations

- a. An expression system should involve an inducing agent (fludeoxyuridine, Methotrexate, thymidine, or other proven inducer) and/or folate deficient medium.
- b. Cells should be banded to insure location of the fragile site at Xq27.3.
- c. Score: 50 cells for males, 100 cells for females.

Note: For positive cases (>2 events), fewer cells may be adequate, while in negative (≤ 1 event) or questionable cases (e.g., atypical Xq appearance), many more cells may be required.

- d. If a very low positive result is obtained, one of the following procedures may be used: (1) If one or a small number of positive cells is observed, it is recommended that a second blood sample be requested and/or that multiple induction methods be used; or (2) If only one positive cell is observed, score an additional 100 cells. If more than one cell but less than 3% of metaphases show the fragile X, the result should be regarded as equivocal and another blood sample requested. It may be necessary to request cessation of dietary folate supplementation, i.e., multivitamins.

Note: The proband's phenotype and the family history may need to be taken into account when considering whether or not to request a repeat sample for patients whose results are neither completely negative nor clearly positive.

- e. Fragile X studies should be accompanied by a regular constitutional chromosome analysis. Those 15–20 cells can be included in the total number scored.

- f. If a laboratory reports several consecutive negative studies in patients with a positive fragile X phenotype and/or positive family history, it is suggested that the culture system be checked by either splitting fragile X samples with a laboratory known to have success with the technique or by obtaining cells from a known fragile X positive individual to use as a control.

Note: It is the opinion of some that the scoring of other folate sensitive fragile sites (e.g., 2q13, 2q31, 3p14, 6q23, 6q26, 7p11, 7q32, 8q22, 9p21, 9q32, 10q23, 11q13, 11q23, 12q13, 16p12, 16q22, Xp22) can serve as an internal quality control and that failure to express these sites may be indicative of a deficiency in the expression system.

Cancer Chromosome Studies

Tissues. Bone marrow, peripheral blood (stimulated and unstimulated), tumor, lymph node, effusion, spinal fluid, etc. In cancer cytogenetics, it is important to examine the appropriate tissue, specifically examining those cells that are believed to be cancerous; this will reduce the likelihood of false negative results.

Count. Counting chromosomes without analyzing them is not recommended for cancer specimens.

Analyze. 15–20 cells, if possible. Carefully analyze every chromosome and every possible chromosome band in all cells in order to appropriately identify chromosomally abnormal clones. While the goal should be to analyze 20 or more metaphases, when that number is not available, clinically useful results can often be obtained from considerably fewer than 20 metaphases; in some cases, even a few cells can be important if they demonstrate a specific structural abnormality [e.g., t(9;22) or t(8;14)] that is consistent with the diagnosis. It is important to realize that, since chromosome morphology in neoplastic cells may be poor while normal cells in the same specimen show good morphology, analysis should be performed on cells with varying degrees of quality.

Karyotype. A minimum of 2 cells (of the 15–20) with a minimum of 1–2 per clone; select those cells that demonstrate the major abnormalities.

Evaluation and Interpretation of Mosaicism

The observation of a small number of abnormal cells in the initial cytogenetic analysis may require the examination of additional cells, in order to distinguish between true mosaicism and artifact. Recognizing that the course of action to be taken is dependent upon the specific abnormality observed, the present guidelines make no recommendations regarding the actual number of cells to examine but advise that the following approach be taken. Each laboratory should establish written guidelines for procedures to follow for each general type of abnormality (hypodiploidy, hyperdiploidy, and structural abnormality) and for some of the specific abnormalities (e.g., +2, +16, +21, loss of sex chromosome). These guidelines should be based on current knowledge of the potential clinical significance of particular chromosome abnormalities and reports in the literature of single or multiple abnormal cells, as observed in various types of tissues. Before the significance of small numbers of abnormal cells in an otherwise normal cytogenetic analysis can be understood, more research in this area needs to be done.

In evaluating mosaicism in amniotic fluid cultures, it is always necessary to find

the same aberrant cell line in more than one culture in order to rule out cultural artifacts. When using the in situ analysis method, partial-colony mosaicism is usually interpreted as pseudomosaicism due to in vitro cultural artifact [2], or the aggregation of multiple cells forming a single “focus” or colony.

Chromosome Band Resolution

Chromosome banding resolution should be appropriate to the case and the type of tissue studied. The ISCN (1985) standard system of nomenclature for human chromosomes [3] should be adhered to, and in each study, every chromosome pair should be analyzed band-for-band at least once. Each laboratory should establish protocols clearly defining standards for band resolution for its own cases; these protocols should also address the consequences of an inadequate study, i.e., signed out, repeated, or diagnosis deferred. In addition, the laboratory director should define the goals for band resolution and monitor the progress of the laboratory toward meeting these goals.

The 400 band resolution level is a reasonable minimal goal for most specimens, particularly amniotic fluid. In certain circumstances, such as peripheral blood studies of children with mental retardation, dysmorphic features, or birth defects, or of couples with spontaneous abortions, the goal should be a band resolution of 550 or greater. On the other hand, useful cytogenetic information can sometimes be obtained at band levels lower than 400; in cancer cytogenetics, e.g., several factors, including the patient's disorder, the quality and quantity of the specimen, and the treatment received prior to sampling, can adversely affect the quality of the chromosomes, so that it is not possible to achieve a 400 band level of resolution.

GENERAL RECOMMENDATIONS

General Cytogenetic Laboratory Recommendations

1. Chromosome preparations should be banded, using G, Q, or R techniques; other staining methods should be available at the discretion of the laboratory director (C-banding, NOR, etc.). Nonbanded studies alone are not recommended, but can be useful in specific situations, e.g., mitomycin C stress tests, breakage studies, and evaluation of satellites.
2. Handling of specimens during culturing and harvesting procedures (except centrifugation, where tubes should be capped with seal-forming screw tops) should be performed in a biological safety cabinet (vertical laminar flow hood), minimum Class II A or B, until the cells are in fixative. This helps to protect both the culture and the laboratory worker.
3. Duplicate or independently established cultures are recommended for all specimen types.
4. Chromosome nomenclature should conform to those standards published by the Standing Committee on Human Cytogenetic Nomenclature (ISCN 1985) [3].
5. Each laboratory should have established written guidelines regarding:
 - a. Logging in of specimens and reporting of results
 - b. Testing of media and sera for sterility and growth potential
 - c. Equipment maintenance and quality control monitoring
 - d. Safety proceduresA complete guide to these issues can be found in the *ACT Cytogenetics Laboratory Manual* [1].
6. Maintenance of records, slides, negatives, and reports should conform to state

laws, or to guidelines established by local, regional, or national certifying or regulatory agencies.

7. Laboratories should participate in a cytogenetic proficiency testing program, such as those offered by New York State, CAP, and/or the regional genetics networks (e.g., SERGG, PacNoRGG, MSRGN, PSRGN, and NERGG).
8. It is recommended that the supervisor and those technologists who are eligible be certified by the NCMLP as Clinical Laboratory Specialists in Cytogenetics, and that they maintain that certification through CEUs or re-examination.

Additional Recommendations for Amniotic Fluid (AF) and Chorionic Villus Sampling (CVS)

1. Two or more independent cultures should be set up from amniotic fluid specimens. Cultures from each patient should be split between two incubators with independent electrical circuits, back-up CO₂ sources, and emergency alarms; the two flasks or dishes should be harvested independently. Amniotic fluid and CVS cultures should be grown in incubators separate from those used for other types of tissue (e.g., peripheral blood, bone marrow, products of conception, tumors).
2. Incubate all clinical human cell cultures in an incubator separate from those containing nonhuman cultures, or transformed or established cell lines.
3. Success Rate for Amniotic Fluid: It is recommended that there be a minimum chromosome analysis success rate (i.e., adequate number of banded karyotypes) of 95% of the adequate specimens, based on a consecutive 3-month average. This calculation does not include infrequent mechanical accidents or malfunctions. Records should be kept regarding the cause for each failure and the remedies taken to prevent future failures.

Verification of Results in Prenatal Diagnosis

Normal results should be documented by pregnancy outcome. Abnormal prenatal diagnostic results should be confirmed, to the extent possible, by second culture and analysis, at birth or termination. A sample of amniotic fluid, obtained for culture prior to the induction of termination, can also be used to confirm results. When mosaicism is to be evaluated, it may be necessary to examine multiple tissues.

Turnaround Times

The following are goals for reasonable turnaround times for various specimens. However, it is not appropriate to achieve rapid results at the expense of optimal quality.

1. Amniotic fluid and chorionic villi: Ninety percent of the specimens should be reported within 21 days, preferably within 14 days. The decision to repeat an amniocentesis should be made within 10–14 days of the initial amniocentesis.
2. Peripheral blood: Written results should be reported within 4 weeks, preferably within 2 weeks.
3. Stat peripheral blood, newborn bone marrow, or cord blood: Final reports should be sent within 7 days.
4. Neoplastic bone marrow and peripheral blood: Final reports should be sent within 4 weeks.

GLOSSARY

Count (verb): To enumerate the number of chromosomes in a cell.

Analyze (verb): To evaluate each chromosome in a cell, comparing the homologues band-for-band, either through the microscope or using a photomicrograph.

Karyotype (verb): To arrange the chromosomes of a single cell in the standard arrangement according to size, centromere location, and banding pattern; in these guidelines, a karyotype is either produced by cutting and arranging the chromosomes from a photograph, or using an automated image analyzer.

Score (verb): To examine a given number of cells for particular chromosomal phenomena such as: (a) the number of sister chromatid exchanges per cell, (b) the presence and incidence of aberrations, including breaks, gaps, fragile sites, triradial and quadriradial figures, etc., and (c) the presence of a specific chromosomal rearrangement.

Clone: A population of cells derived from a single progenitor cell, having the same or related chromosome complement. Three cells with the same missing chromosome, or two cells with the same extra chromosome or structurally abnormal chromosome, constitute criteria for a clone.

High Resolution Chromosome Analysis: The use of elongated and finely banded prometaphase or late prophase chromosomes, displaying a minimum of 550 distinct bands or subbands, to detect minute chromosomal defects. Such chromosome preparations usually require the utilization of cell synchronization or other special techniques.

Mosaicism: The presence of two or more chromosomally distinct cell lines. In prenatal diagnosis studies, each cell line must be observed in more than one independent culture.

Pseudomosaicism: In prenatal diagnosis, a single cell, a cell line, or cell lines confined to a single independent culture. Using the in situ colony method, aberrant cells that are clearly confined to part of a single colony may be considered to be pseudomosaic. Whole colony aberrations confined to a single culture vessel may also be interpreted as pseudomosaic.

Culture Failure: Any situation requiring a repeat sample. Culture failure can be due to insufficient growth or laboratory-induced contamination, ambiguity regarding patient identification, etc. Failures caused by conditions prior to laboratory receipt of the sample (e.g., frozen, contaminated, clotted, hemolyzed, or delayed specimens) are not considered to be culture failures.

Independent Cultures: Cultures that originate from different primary cultures.

Primary Culture: A culture that has never been subcultured.

Cell Line: A group of cells from an individual that have the same karyotype.

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